

Masking and unmasking of the sialic acid-binding lectin activity of CD22 (Siglec-2) on B lymphocytes

(CD22/Siglecs/B cells/sialidase/sialic acids)

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ABSTRACT CD22 is a B cell-restricted glycoprotein involved in signal transduction and modulation of cellular activation. It is also an I-type lectin (now designated Siglec-2), whose extracellular domain can specifically recognize α 2-6-linked sialic acid (Sia) residues. This activity is postulated to mediate intercellular adhesion and/or to act as a coreceptor in antigen-induced B cell activation. However, studies with recombinant CD22 indicate that the lectin function can be inactivated by expression of α 2-6-linked Sia residues on the same cell surface. To explore whether this masking phenomenon affects native CD22 on B cells, we first developed a probe to detect the lectin activity of recombinant CD22 expressed on Chinese hamster ovary cells (which have no endogenous α 2-6-linked Sia residues). This probe is inactive against CD22-positive B lymphoma cells and Epstein-Barr virus-transformed lymphoblasts which express high levels of α 2-6-linked Sia residues. Enzymatic desialylation unmasks the CD22 lectin activity, indicating that endogenous Sia residues block the CD22 lectin-binding site. Truncation of the side chains of cell surface Sia residues by mild periodate oxidation (known to abrogate Sia recognition by CD22) also had this unmasking effect, indicating that the effects of desialylation are not due to a loss of negative charge. Normal resting B cells from human peripheral blood gave similar findings. However, the lectin is partially unmasked during *in vitro* activation of these cells. Thus, the lectin activity of CD22 is restricted by endogenous sialylation in resting B cells and may be transiently unmasked during *in vivo* activation, perhaps to modulate intercellular or intracellular interactions at this critical stage in the humoral response.

CD22, a B cell-specific molecule which appears on the cell surface at the pre-B cell stage, is thought to function as a modulator of intracellular signaling through the B cell receptor (sIgM) complex and as a cell surface adhesion molecule (1–3). The cell-binding properties of CD22 have been investigated using either a soluble chimeric form of CD22 containing the three amino-terminal Ig-like domains of human CD22 fused to the Fc domains of human or mouse IgG (CD22-Rg), or with full-length CD22 expressed in monkey COS cells or Chinese hamster ovary (CHO) cells (4–13). When induced to express full-length CD22 by cDNA transfection, these cells can adhere to a variety of others, including erythrocytes, activated B and T cells, accessory cells, and endothelial cells. A series of sialoglycoprotein ligands on activated T and B cells are recognized by recombinant CD22, including CD45, a major leukocyte tyrosine phosphatase (4, 7, 14, 15). The common terminal structural motif of N-linked glycans Sia α 2-6Gal β 1-4GlcNAc β 1- (the α 2-6-sialyllactosaminyl group) was found to

be the recognition target for all forms of CD22-mediated adhesion (4, 7, 14). This sequence is the product of action of the Golgi sialyltransferase ST6Gal I (Gal β 1-4GlcNAc-specific α 2-6-sialyltransferase), which is itself highly regulated in a variety of cell types including lymphocytes (16, 17), endothelial cells (12, 18), and hepatocytes (19, 20). Indeed, some cell surface epitopes which give specific staining patterns in lymphoid tissues (e.g., CD75 and CD76) also depend on the expression of ST6Gal I (21, 22). The discovery of its carbohydrate-binding lectin activity led to the classification of CD22 as an Ig-type (I-type) lectin (23), along with some other cell surface molecules that specifically bind sialic acid, including sialoadhesin, myelin-associated glycoprotein, and CD33 (8, 23–27). In all of these molecules, which have recently been named the Siglecs (28), the sialic acid-binding property is mediated primarily by the amino-terminal V-set Ig domain, with some contribution by the next C2-set domain (13, 29).

Several groups produced mice genetically defective in the expression of CD22 (30–32). Despite some variability in the specific phenotypes reported, the common theme consists of altered cellular responses to sIgM ligation and changes in the humoral responses to T cell-dependent and/or T cell-independent antigens (30–33). Taken together with the known association of CD22 with the B cell receptor (34, 35) and with certain cytosolic tyrosine phosphatases (34, 36–39), it is reasonable to conclude that CD22 plays an important role in “tuning” the B cell response to different types and levels of antigenic challenge (1–3). However, the function of the highly specific lectin activity of CD22 remains uncertain. The primary target sequence of the lectin (the α 2-6-sialyllactosaminyl group) is in abundance on the large variety of sialoglycoconjugates of various cell surfaces and in physiological fluids (11, 40). Therefore, the lectin function of CD22 must be regulated at different stages of normal B cell function to prevent undesirable CD22-mediated interactions with inappropriate targets. In this regard, we and others previously reported that the lectin function of recombinant CD22 can be abrogated by 9-O-acetylation of sialic acids on ligands (9), by the simultaneous expression of the ST6Gal I enzyme in the same cell (12, 15, 41), or by sialylated N-glycans carried by the CD22 molecule itself (42, 43). Since then, the latter form of masking by endogenous ligands on the same cell surface has been reported for the lectin activities of recombinant forms of three other

This paper was submitted directly (Track II) to the *Proceedings* office. Abbreviations: PAA, polyacrylamide; PAA-B, PAA substituted with biotin; 6’PAA, PAA substituted with α 2-6-sialyllactose; 3’PAA, PAA substituted with α 2-3-sialyllactose; Sia, sialic acid; CD22-Rg, three amino-terminal Ig-like domains of human CD22 fused to Fc domains of human or mouse IgG; ST6Gal I, Gal β 1-4GlcNAc-specific α 2-6-sialyltransferase; AUS, *Arthrobacter ureafaciens* sialidase; PBMC, peripheral blood mononuclear cell; Tc, Tricolor fluorophore, FITC, fluorescein isothiocyanate; PE, phycoerythrin; SA-PE, streptavidin conjugated with PE; PWM, pokeweed mitogen; PMA, phorbol ester. *To whom reprint requests should be addressed.

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Siglecs: sialoadhesin, myelin-associated protein, and CD33 (43–46).

These data suggesting the regulatory effects of ligands on the same cell surface were all obtained with *in vitro* cell adhesion assays utilizing transfected cell lines. Is this an artifact of the transfected cell systems used, or does the same type of regulation actually occur *in vivo* on native cells? If the latter were true, it would significantly constrain the plausible hypotheses concerning the function of the CD22 lectin. However, the lectin function has been difficult to demonstrate directly on cell surfaces without a specific soluble probe.

MATERIALS AND METHODS

General Chemicals and Biologicals. These chemicals and biologicals were mostly from Sigma or Oxford Glycosystems (Rosedale, NY). The others were as follows: pokeweed mitogen (PWM) and phorbol ester (PMA) from GIBCO/BRL; ionomycin from Calbiochem; lipopolysaccharide–*Escherichia coli* serotype 055:B5 from Fluka; and Hy-Q media from HyClone.

Antibodies and Probes. HB22-7 (adhesion-blocking) and HB22-27 (nonblocking) anti-CD22 mAbs were gifts from Dr. Thomas Tedder (Duke University, Durham, NC). Other reagents used were as follows: T-015, a pan-B cell anti-CD22 antibody (Dako), fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG secondary antibody and anti-human CD40 mAb (NA/LE grade, PharMingen), FITC or Tricolor-conjugated anti-human CD22 and CD19 mAbs (Caltag Laboratories, Burlingame, CA), phycoerythrin-conjugated streptavidin (SA-PE) and anti-human IgM (Fab')₂ fragments for B cell activation (Jackson ImmunoResearch Laboratories), FITC or biotin-conjugated polyacrylamide substituted with α 2–6-sialyllactose (6'PAA-FITC and 6'PAA-B, respectively, its analog with α 2–3 Sia substitution, (3'PAA-B), a nonsialylated version (Lac-PAA), and nonbiotinylated forms of all conjugates were from GlycoTech (Rockville, MD).

Cell Lines. Parental CHO-K1 and CHO cells stably transfected with full-length human CD22 cDNA (12) were cultured in α -MEM supplemented with 10% fetal calf serum and L-glutamine. The B lymphoma cell line (Daudi) and Epstein-Barr virus-transformed human B cells (gift from Peter Parham, Stanford University, CA) were cultured in RPMI 1640 medium with 10% fetal calf serum and L-glutamine.

Peripheral Blood Mononuclear Preparations. Fresh peripheral blood samples were obtained from normal human volunteers and mononuclear cells separated by Ficoll density gradient, diluted 1:1 with serum-free RPMI medium, followed by two washes with the same medium.

Sialidase Treatments. Cells were resuspended in serum-free RPMI medium (1.5×10^6 cells/ml) containing 0.05 M Hepes (pH 6.9) and incubated for 15 min at room temperature (RT) with 20 milliunits of *Arthrobacter ureafaciens* sialidase (AUS). Excess sialidase was removed by washing several times with serum-free RPMI followed by washing with the staining buffer.

Mild Periodate Treatment. Cells were washed with PBS and resuspended (1.5×10^6 cells/ml) in phosphate buffer (pH 7.4) containing freshly dissolved 2 mM NaIO₄ and incubated for 30 min at 4°C in the dark. Excess periodate was destroyed by adding 10 μ l of 20% glycerol followed by immediate washing with staining buffer.

Activation of B Cells. Peripheral blood mononuclear cells (PBMCs) were resuspended in RPMI (1×10^6 cells/ml) supplemented with 10% heat-inactivated fetal calf serum, 1% L-glutamine, 5×10^{-5} M 2-mercaptoethanol, and 1% Pen-strep (complete RPMI) and cultured at 3 ml/well in a 12-well Corning plate. Cells were activated with 5 ng/ml PMA and 2 μ M ionomycin, PWM at 10 μ l/ml (per manufacturer's instruction), lipopolysaccharide at 15–150 μ g/ml final concentration, or by mouse monoclonal anti-human IgM (Fab')₂ fragments (10 μ g/ml), with or without mouse monoclonal antihuman CD40 (10 μ g/ml). Cells from each well (3×10^6) were

removed at different times for the flow cytometry analysis to check the presence of CD22 and to probe its lectin activity. Cell proliferation was monitored by [³H]thymidine incorporation.

Flow Cytometric Analysis. Flow cytometry was performed on a Becton Dickinson FACscan machine. CHO-K1 and CD22-transfected CHO cells were first detached from plates by incubation with PBS and 0.25 mM EDTA at RT. Cells ($0.2-1 \times 10^6$) were washed three times with ice-cold PBS containing 0.02% sodium azide and 1% BSA (staining buffer) and incubated for 1 h in 100 μ l of the same buffer containing 1:100 of different anti-CD22 mAbs used according to the experimental design. Nonconjugated antibodies were detected with FITC-conjugated goat anti-mouse IgG secondary antibody. The lectin activity of cell surface CD22 was examined after several washings with staining buffer by incubating the cells (intact or with sialidase or periodate treatment) in 100 μ l of staining buffer containing 1–1.5 μ g 6'PAA-B probe for 1 h in ice (conditions of time and probe concentration were determined to be optimal). After washing once with 0.5 ml of the staining buffer, cells were incubated with PE-conjugated streptavidin for 30 min to detect binding of the biotinylated probe.

RESULTS AND DISCUSSION

A Probe Carrying Multiple 6'-Sialyllactosyl Groups Can Detect Lectin Activity on the Surface of CHO Cells Transfected with Recombinant CD22. We wished to use flow cytometry to directly probe the lectin-binding site of CD22 on the cell surfaces. Each CD22 molecule only has one binding site for sialic acid, and the affinity of CD22 for the minimal ligand Sia α 2–6Gal β 1–4Glc is ~ 30 μ M (47), which is unlikely to survive the washing steps involved in preparing cells for flow cytometry. However, previous studies have shown that the CD22 molecules on cell surfaces can be present in homomultimeric complexes (47), which should bring several binding sites into close proximity to one another. Indeed, we previously found one batch of commercial α 1-acid glycoprotein that could weakly detect recombinant CD22 lectin activity on cell surfaces (47). However, binding was not strong, and subsequent batches did not reproduce this result, apparently because the density of 2–6-linked Sias was not high enough (L. D. Powell and A.V., unpublished observations). We therefore tried the two previously identified high-affinity serum ligands for CD22: soluble IgM and haptoglobin (11). Although some positive results were obtained (data not shown), use of these probes was limited by the concern that endogenous molecules of similar type may be already present on the surface of B cells isolated from human blood and by the low-level cross-reactivity of secondary antibodies against other cell surface components of freshly isolated human cells. Also, it is difficult to rule out binding involving other aspects of these natural human glycoproteins. Finally, it is somewhat difficult to design negative controls for these probes. For a more reliable and reproducible probe, we turned to a commercially available synthetic conjugate of biotinylated polyacrylamide substituted with multiple copies of Sia α 2–6Gal β 1–4Glc β 1 (6'PAA-B) to probe the lectin activity of cell surface CD22 [Glc can substitute for GlcNAc, without affecting binding (47)].

Positive control experiments were performed with CHO cells transfected with recombinant CD22. CHO cells themselves do not express the α 2–6-sialyllactosaminyl group (11). Preliminary experiments suggested that the binding of 6'PAA-B to the CD22 lectin site was partly dependent on the density of CD22 on the cell surface (data not shown). Stably transfected cells expressing a high level of CD22 were therefore selected by cell sorting and used in the subsequent experiments. As shown in Fig. 1, 6'PAA-B did not bind to the wild-type CHO-K1 cells (Fig. 1A), whereas it bound to CD22-transfected CHO cells (Fig. 1B). A conjugate of Gal β 1–4GlcNAc β 1-PAA did not bind to the latter, indicating the requirement for Sia residues (data not shown). Likewise, 3'PAA-B, in which PAA-B is substituted with α 2–3- rather than α 2–6-linked Sias, did not bind to the CD22-transfected cells (Fig. 1B). Also, mild periodate oxidation of 6'PAA-B, which selectively

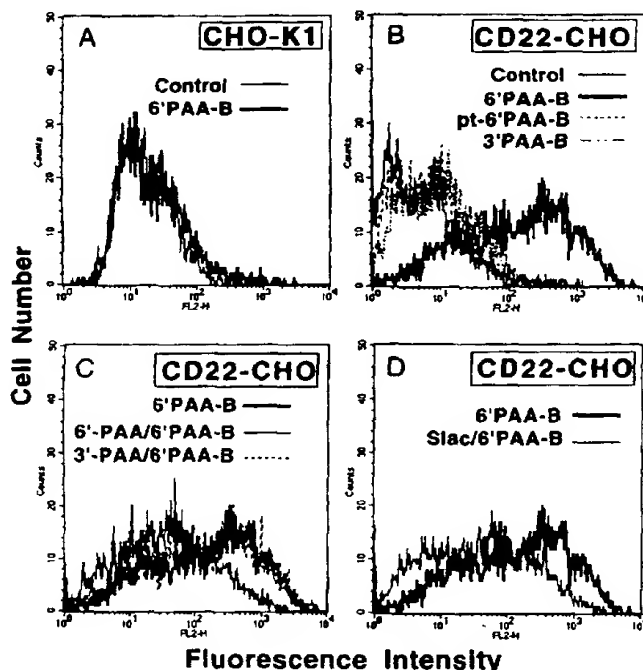


FIG. 1. Detection of cell surface CD22 lectin activity on transfected CHO cells. Wild-type or CD22-transfected CHO cells were probed with the biotinylated polyacrylamide polymers in the presence or absence of inhibitors. Binding was detected using SA-PE and single-color flow cytometry. Negative control incubations had SA-PE alone. (A) Parental CHO-K1 incubated with and without 6'PAA-B; (B) CD22-CHO cells incubated with 6'PAA-B, mild periodate-treated (pt) 6'PAA-B, or 3'PAA-B; (C) competition of 6'PAA-B binding to CD22-CHO cells with a fivefold excess of nonbiotinylated 6'PAA or 3'PAA; (D) competition of 6'PAA-B to CD22-CHO cells with 1 mM α 2-6-sialyllactose (Slac).

truncates the side chain of the Sia residues, abrogated binding of this probe to CD22-transfected cells (Fig. 1B). In competition assays, nonbiotinylated 6'PAA competed with 6'PAA-B for binding, whereas 3'PAA did not (Fig. 1C). Finally, 1 mM α 2-6-sialyllactose partially inhibited binding of the probe to CD22-transfected cells (Fig. 1D), complete inhibition is sometimes difficult to achieve when a monovalent-soluble ligand is competing with a multivalent one. These results indicate that 6'PAA-B can be used as a specific probe for detecting unoccupied CD22 lectin molecules on the surface of cells.

Desialylation or Truncation of the Side Chains of Cell Surface Sialic Acids Unmasks CD22-Lectin Activity on B Lymphoma Cells and on Epstein-Barr Virus-Transformed B-Lymphoblastoid Cells. The 6'PAA-B probe was used to detect lectin activity on a B lymphoma cell line, Daudi, which expresses cell surface CD22 (Fig. 2A). These cells, unlike CHO cells, also express a high level of α 2-6-sialyllactosaminyl groups (21, 47) which could potentially mask the lectin function of CD22. Indeed, neither 6'PAA-B or 3'PAA-B gave obviously detectable binding (Fig. 2B), despite the high density of CD22 molecules on the cell surface (Fig. 2A). One explanation is that the lectin-binding site of CD22 is occupied by endogenous ligands. Indeed, desialylation of the cells by sialidase (Fig. 2C) markedly increased binding of 6'PAA-B, but not 3'PAA-B, indicating unmasking of the lectin activity. A similar result was obtained by truncating the side chains of the cell surface sialic acids by mild periodate oxidation (Fig. 2D). The binding of 6'PAA-B was also inhibited by high concentrations of α 2-6-sialyllactose (data not shown). Competition experiments with antibodies confirmed the specific binding of 6'PAA-B to CD22 molecules on these cells (Fig. 3). An anti-CD22 mAb (HB22-7) which blocks CD22-mediated cell-cell interactions (5) partially blocked the binding of 6'PAA-B to sialidase-treated Daudi cells (Fig. 3D). Conversely, preincubation of the sialidase-treated cells with 6'PAA-B partially blocked the

binding of this antibody to CD22 (Fig. 3C). This competition was not seen with another anti-CD22 mAb (HB22-27) which does not block CD22-dependent cell adhesion (data not shown). As seen in Fig. 3 (C and D), the order of addition of the probe and blocking antibody affected the exact binding profile obtained. The incomplete competition seen could be due to either lack of saturation of binding sites or closely adjacent binding sites of the probe and the antibody. Regardless, these results not only confirm the ability of 6'PAA-B to detect the lectin activity of naturally occurring CD22 but also show that in cells expressing α 2-6-sialyllactosaminyl groups, the lectin site of CD22 is masked by endogenous ligands on the same cell surface or on CD22 itself. The data also indicate that mild periodate oxidation of the cells gives a similar result to sialidase with regard to unmasking of lectin activity. The latter result not only provides an independent confirmation of the importance of cell surface sialic acids, but also ensures that the results with sialidase are not due to a loss of cell surface negative charge. To assure that this masking was not an unusual phenomenon seen only in malignant lymphoma cells, the lectin activity of CD22 was also probed with 6'PAA-B on Epstein-Barr virus-transformed CD22⁺ human B lymphoblastoid cells. As with the Daudi cells, the probe was inactive against the native cells, and treatment of the cells with either sialidase or periodate unmasked the lectin binding sites (data not shown).

Resting CD22-Positive B Cells in Human Peripheral Blood also Have Masked Lectin Activity. The lymphoma and lymphoblastoid cell lines are, by definition, somewhat activated and constantly expanding in culture. Furthermore, activated B cells are known to express endogenous ST6Gal I at high levels (16). From the physiological point of view, it is important to know the status of the CD22 lectin on resting human B lymphocytes prior to their activation. The 6'PAA-B was therefore used to probe resting peripheral blood B cells from normal human volunteers. To study the cells in as native a

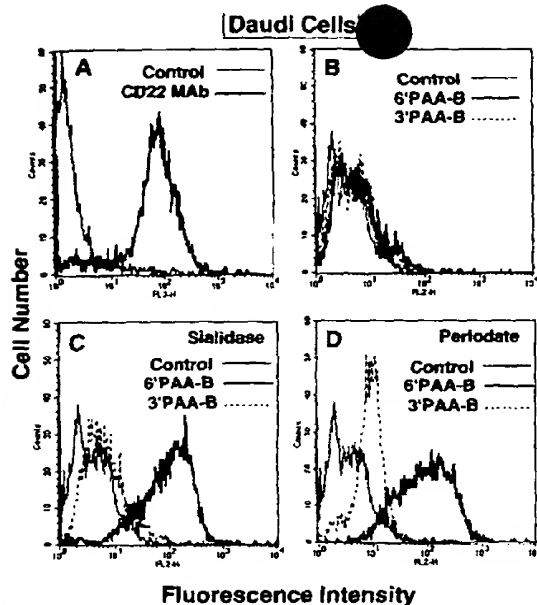


FIG. 2. Sialidase or periodate treatment of B lymphoma cells unmasks lectin activity. Cultured Daudi B lymphoma cells were washed and then probed as indicated. (A) CD22 detected by T-015 anti-CD22 mAb and FITC-conjugated goat anti-mouse IgG; (B) lectin activity of intact cells probed by 6'PAA-B or 3'PAA-B; (C) lectin activity probed after sialidase (AUS) treatment; (D) lectin activity after mild periodate treatment. Probe binding in B–D was detected using SA-PE. Control incubations contained the secondary reagent alone.

condition as possible, no attempt was made to fractionate the B cells from the total peripheral blood mononuclear cells. However, the mixture of cells was washed well and preincu-

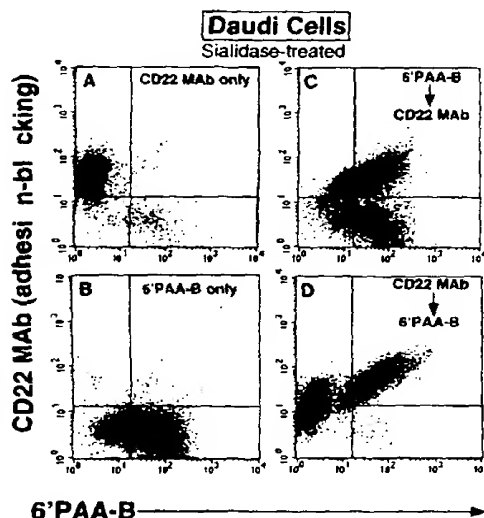


FIG. 3. Partial competition by an adhesion blocking anti-CD22 mAb. Cultured Daudi B lymphoma cells were washed, AUS treated, and then probed as described in the legend to Fig. 2. (A) Incubation with the HB22-7 adhesion-blocking mAb alone; (B) incubation with 6'PAA-B alone; (C) incubation with 6'PAA-B first followed by HB22-7 antibody; and (D) incubation with HB22-7 antibody first followed by 6'PAA-B.

bated for 30 min at RT in serum-free medium to remove any serum proteins that inhibit CD22 binding. Analysis showed that the lectin-binding sites of CD22 on these normal resting human B cells are also completely masked by endogenous ligands. As shown in Fig. 4, sialidase treatment is needed to expose the binding sites. The sialidase treatment was performed under mild conditions (15 min at RT) to limit the possibility of activation or mobilization of cell surface molecules. The adequacy of removal of Sia in this reaction was confirmed by observing the destruction of binding sites for CD22-Rg (data not shown). Several different sialidases were also noted to be almost equally active in exposing the lectin-binding sites on blood cells (data not shown). Since CD22-positive cells are a minority of peripheral blood mononuclear cells, double-color flow cytometry was used to show almost all of the circulating CD22-positive cells (Fig. 5) expressed some 6'PAA-B-binding sites after sialidase treatment (another unrelated population of CD22-negative cells also bound the probe, indicating the presence of a novel lectin(s); this matter is being further investigated). Mild periodate oxidation of 6'PAA-B demonstrated the requirement for intact Sias for recognition (Fig. 5B). We conclude that, as with the lymphoma and lymphoblastoid cells, the CD22 molecules on the surface of resting circulating B cells are occupied by endogenous ligands from the same cell surface and/or of plasma origin.

The CD22 Lectin-Binding Site Can Be Unmasked During Activation of Normal B Cells. When recombinant CD22 is expressed on the surface of transfected CHO or COS cells, it can be shown in many *in vitro* assays to mediate adhesion with various cell types, including activated endothelial cells, other B cells, erythrocytes, and T cells. In the last instance, it has been suggested that CD22 and its cytosolically associated tyrosine phosphatase SHP-1 may be sequestered away from membrane Ig through interactions with counterreceptors on T cells (37). It has also been noted that a major potential ligand for CD22 on T cells is the tyrosine phosphatase CD45 (4, 15), whose sialic acid residues are almost exclusively in α 2-6 linkage (48). Another suggested *in vivo* interaction is the binding of circulating B cells to activated endothelium, which shows selective up-regulation of the Sia α 2-6 linkage (12, 18). However, the data presented above indicate that resting B cells cannot participate in any of these proposed interactions, because the lectin-binding site is masked. We therefore asked whether the lectin function might become

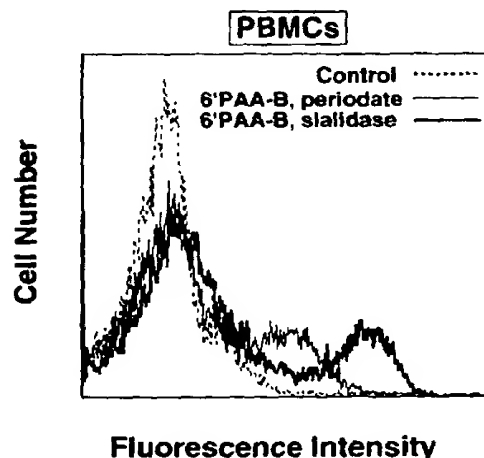


FIG. 4. Unmasking of 6'-sialyllactosamine lectin function in human PBMCs cells by sialidase or mild periodate treatment. PBMCs were washed well, preincubated in serum-free medium for 20 min at RT, and then treated with either AUS, mild periodate, or a buffer control (PBS) before being probed by 6'PAA-B followed by SA-PE.

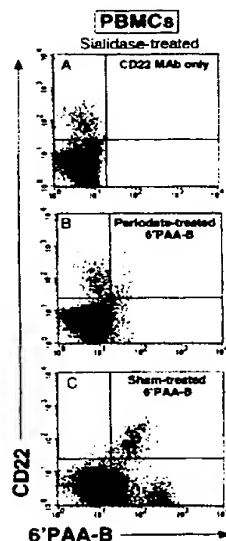


FIG. 5. Some of the 6'PAA-binding sites exposed by sialidase are on CD22-positive PBMCs. Sialidase-treated PBMCs were incubated with nonblocking anti-CD22-Tc mAb (A), anti-CD22-Tc mAb plus mild periodate-treated 6'PAA-B (B), or anti-CD22-Tc mAb and 6'PAA-B (C). Detection of probe binding was by flow cytometry after incubation with SA-PE.

unmasked during activation of B cells, when such interactions might be most appropriate (1–3). Human PBMCs were subjected to several different treatments known to activate B cells. Activation with PMA (protein kinase C activation) and ionomycin (calcium ionophore) results in partial unmasking of 6'PAA-binding sites on CD22-positive cells (data not shown). Interestingly, the maximum degree of unmasking was seen on the larger CD22⁺ blast cells arising from the activation. Activation with PWM, or with bacterial lipopolysaccharide, also results in varying degrees of unmasking of 6'PAA-binding sites of B cells (data not shown). The lectin function exposed through these activation methods was detectable at different time intervals from 3 h up to 2 days (data not shown).

To mimic a more natural activation state for B cells, anti-human IgM (Fab')₂ fragments were used to cross-link the B cell receptor on the PBMCs. No exposure of the CD22 lectin activity was seen under these circumstances, which also did not lead to vigorous proliferation of the cells, as monitored by the [³H]thymidine incorporation assay (data not shown). However, anti-IgM along with anti-human-CD40 gave good proliferation and partially unmasked the CD22 lectin sites. This could be detected in as little as 30 min after activation. Fig. 6 shows an example of the exposure of the lectin sites on CD22-positive cells 1.5 h after activation with anti-IgM/anti-CD40. Unmasking of the lectin sites was also observed with anti-CD40 alone (data not shown), even though the degree of overall cell proliferation was at least threefold lower than that seen with anti-IgM plus anti-CD40. Unlike the unmasking of the lectin site through activation with more artificial procedures (PMA/ionomycin, PWM, and lipopolysaccharide), lectin exposure following activation with anti-IgM/anti-CD40 did not last long and was often not detectable after several hours of activation. This could either be due to remasking or the gradual internalization of CD22 itself by endocytosis, a previously well-described phenomenon (49). The density of CD22 on the cell surface did indeed decrease 3 h after activation (data not shown).

CONCLUSIONS AND PERSPECTIVES

We have shown that a biotinylated, α 2–6-sialyllactose-substituted polyacrylamide can specifically probe the lectin activity of cell

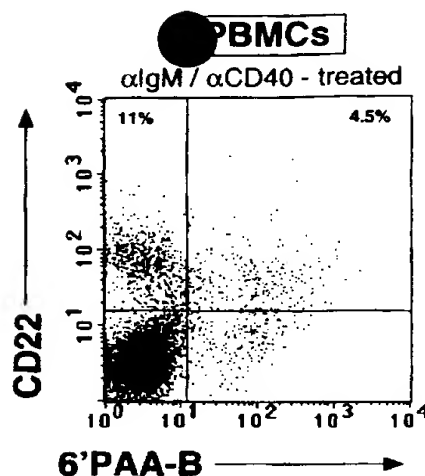


FIG. 6. Unmasking of CD22 lectin sites on PBMCs by anti-IgM and anti-CD40 antibodies. PBMCs were resuspended in complete RPMI, incubated with anti-IgM and anti-CD40 antibodies as described in *Materials and Methods*, and analyzed at different time points by double-color flow cytometry using anti-CD22-Tc mAb and 6'PAA-FITC. These data are from an example of a 1.5-h time point, when exposure tended to be maximal.

surface CD22. These sites were masked in B lymphoma cells, Epstein-Barr virus-transformed B lymphocytes, and mature resting human peripheral blood B cells. Although the nature of the endogenous blocking ligands for CD22 remains to be determined, they could include sialylated N-glycans carried by the CD22 molecule itself (42, 43) and other cell surface glycoproteins such as CD45 (15). In this regard, it is interesting that a small fraction of the cell surface CD22 is associated noncovalently with the membrane IgM molecule (34, 35). With circulating B cells, the possibility must also be considered that the ligands are adsorbed high-affinity serum glycoproteins such as IgM and haptoglobin (11). In this regard, others have recently reported that haptoglobin can bind to the cell surface of peripheral blood B cells in a manner possibly dependent on CD22 (50). However, we found that incubating cells in serum-free medium for a short time (30 min) did not release any of the occupied binding sites, suggesting that endogenous membrane-bound ligands may be crucial in maintaining the masked state of the lectin. The fact that these ligands can be completely destroyed by short *in vitro* treatments with sialidase or mild periodate oxidation indicates that they are in a dynamic and reversible equilibrium on the cell surface. The failure of the polyvalent solution phase probe to compete with these endogenous sialylated ligands may be because the latter are present at a very high local concentration at the cell surface.

The question arises as to how the lectin unmasking takes place. Although we have yet to perform detailed kinetic studies, it does seem to occur relatively rapidly, raising several possibilities, including a conformational change in CD22 molecules giving improved binding, an induced clustering of CD22 molecules giving enhanced binding of the multivalent probe, conformational or structural changes of endogenous ligands (e.g., CD45 on B cells) which lower their affinity, or the action of an endogenous sialidase removing the cell surface ligands. Regarding the first possibility, there is a precedent for activation-induced enhancement of binding by other leukocyte receptors such as integrins (51). The second possibility needs to be explored by cross-linking experiments. However, it is unclear how the activated or clustered CD22 molecules escape from inhibition by the endogenous ligands. The possibility of conformational changes of endogenous ligands also needs to be studied. The final and most interesting possibility is that

unmasking is due to the action of an endogenous sialidase (the rapidity of the response is somewhat at simple replacement by biosynthesis of new glycoproteins with altered forms of sialic acid linkage). With regard to this possibility, it is interesting that one form of mammalian sialidase is encoded by the *neu-1* gene, which is located within the major histocompatibility complex in both mice and humans (52). Also, previous studies have suggested the existence of a B cell sialidase that can affect T:B cell interactions (53).

Regardless of the mechanism involved, we can also ask when in the life of a B cell this unmasking actually occurs *in vivo*. Although the lectin sites can be unmasked with a variety of different *in vitro* activation methods, the B cell-specific activation with anti-IgM and anti-CD40 is the most physiologically relevant. In this regard, it is interesting that anti-CD40 alone is capable of inducing the exposure. In the *in vivo* setting, B cells that have made contact with helper T cells are the most likely to have their CD40 molecules encounter CD40 ligands (54). Thus, we suggest that the lectin activity of CD22 might be released from endogenous ligands precisely during the intimate association of B cells with T cells (55) that includes the delivery of CD40-mediated signals (54). This could serve the function of either releasing the CD22 from binding to some critical endogenous ligand (e.g., sIgM) and/or exposing it for binding to a critical ligand on the apposing T cell (e.g., CD45). Either of these changes could also alter the clustering and distribution of CD22 and its cytosolic domain which is known to associate with certain tyrosine phosphatases (37–39).

These observations also suggest a general mechanism by which the Siglec family of sialic acid-binding lectins might regulate their exposure and function in the midst of a milieu of natural glycoproteins, many of which carry many copies of their basic ligand structure (11). This provides a way for a receptor with a very common ligand to expose its activity only when it is really needed, perhaps only in a protected milieu, away from circulating soluble inhibitors. Finally, during probing for the CD22 lectin in various cell types, the current studies have detected additional masked sialic acid-dependent binding sites (see for example, Figs. 2, 5, and 6) that cannot be explained on the basis of any previously known sialic acid-binding lectins. We are currently pursuing this matter.

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- Clark, E. A. (1993) *J. Immunol.* 150, 4715–4718.
- Cyster, J. G. & Goodnow, C. C. (1997) *Immunity* 6, 509–517.
- Tedder, T. F., Tuscano, J., Sato, S. & Kehrl, J. H. (1997) *Annu. Rev. Immunol.* 15, 481–504.
- Stamenkovic, I., Sgroi, D., Aruffo, A., Sy, M. S. & Anderson, T. (1991) *Cell* 66, 1133–1144.
- Engel, P., Nojima, Y., Rothstein, D., Zhou, L. J., Wilson, G. L., Kehrl, J. H. & Tedder, T. F. (1993) *J. Immunol.* 150, 4719–4732.
- Powell, L. D., Sgroi, D., Sjöberg, E. R., Stamenkovic, I. & Varki, A. (1993) *J. Biol. Chem.* 268, 7019–7027.
- Sgroi, D., Varki, A., Braesch-Andersen, S. & Stamenkovic, I. (1993) *J. Biol. Chem.* 268, 7011–7018.
- Kelm, S., Petz, A., Schauer, R., Filbin, M. T., Tang, S., De Bellard, M.-E., Schnaar, R. L., Mahoney, J. A., Hartnell, A., Bradfield, P. & Crocker, P. R. (1994) *Curr. Biol.* 4, 965–972.
- Sjöberg, E. R., Powell, L. D., Klein, A. & Varki, A. (1994) *J. Cell Biol.* 126, 549–562.
- Engel, P., Wagner, N., Miller, A. S. & Tedder, T. F. (1995) *J. Exp. Med.* 181, 1581–1586.
- Hanasaki, K., Powell, L. D. & Varki, A. (1995) *J. Biol. Chem.* 270, 7543–7550.
- Hanasaki, K., Varki, A. & Powell, L. D. (1995) *J. Biol. Chem.* 270, 7533–7542.
- Law, C. L., Aruffo, A., Chandran, K. A., Doty, R. T. & Clark, E. A. (1995) *J. Immunol.* 155, 3368–3376.
- Kelm, S., Schauer, R., Manuguerra, J.-C., Gross, H.-J. & Crocker, P. R. (1994) *Glycoconjugate J.* 11, 576–585.
- Sgroi, D., Koretzky, G. A. & Stamenkovic, I. (1995) *Proc. Natl. Acad. Sci. USA* 92, 4026–4030.
- Lo, N. W. & Lau, J. (1996) *Glycobiology* 6, 271–279.
- Baum, L. G., Derbanc, A., Perillo, N. L., Wu, T., Pang, M. & Uittenbogaart, C. (1996) *J. Biol. Chem.* 271, 10793–10799.
- Hanasaki, K., Varki, A., Stamenkovic, I. & Bevilacqua, M. P. (1994) *J. Biol. Chem.* 269, 10637–10643.
- Wang, X. C., Smith, T. J. & Lau, J. T. Y. (1990) *J. Biol. Chem.* 265, 17849–17853.
- Jamieson, J. C., McCaffrey, G. & Harder, P. G. (1993) *Comp. Biochem. Physiol. B* 105, 29–33.
- Bast, B. J. E. G., Zhou, L.-J., Freeman, G. J., Colley, K. J., Ernst, T. J., Munro, J. M. & Tedder, T. F. (1992) *J. Cell Biol.* 116, 423–435.
- Keppeler, O. T., Moldenhauer, G., Oppenländer, M., Schwartz-Albiez, R., Berger, E. G., Funderud, S. & Pawlita, M. (1992) *Eur. J. Immunol.* 22, 2777–2781.
- Powell, L. D. & Varki, A. (1995) *J. Biol. Chem.* 270, 14243–14246.
- Crocker, P. R., Mucklow, S., Bouckson, V., McWilliam, A., Willis, A. C., Gordon, S., Milon, G., Kelm, S. & Bradfield, P. (1994) *EMBO J.* 13, 4490–4503.
- Crocker, P. R. & Feizi, T. (1996) *Curr. Opin. Struct. Biol.* 6, 679–691.
- Kelm, S., Schauer, R. & Crocker, P. R. (1996) *Glycoconjugate J.* 13, 913–926.
- Collins, B. E., Kiso, M., Hasegawa, A., Tropak, M. B., Roder, J. C., Crocker, P. R. & Schnaar, R. L. (1997) *J. Biol. Chem.* 272, 16889–16895.
- Crocker, P. R., Clark, E. A., Filbin, M., Gordon, S., Jones, Y., Kehrl, J. H., Kelm, S., Le Douarin, N., Powell, L. D., Roder, J., Schnaar, R. L., Sgroi, D. C., Stamenkovic, I., Schauer, R., Schachner, M., van den Berg, T. K., Anton van der Merwe, P., Watt, S. M. & Varki, A. (1998) *Glycobiology* 8, v.
- Nath, D., Van der Merwe, P. A., Kelm, S., Bradfield, P. & Crocker, P. R. (1995) *J. Biol. Chem.* 270, 26184–26191.
- O'Keefe, T. L., Williams, G. T., Davies, S. L. & Neuberger, M. S. (1996) *Science (Washington DC)* 274, 798–801.
- Otipoby, K. L., Andersson, K. B., Draves, K. E., Klaus, S. J., Farr, A. G., Kerner, J. D., Perlmuter, R. M., Law, C. L. & Clark, E. A. (1996) *Nature (London)* 384, 634–637.
- Sato, S., Miller, A. S., Inaoki, M., Bock, C. B., Jansen, P. J., Tang, M. L. K. & Tedder, T. F. (1996) *Immunity* 5, 551–562.
- Nitschke, L., Carsetti, R., Ocker, B., Köhler, G. & Lamers, M. C. (1997) *Curr. Biol.* 7, 133–143.
- LePrince, C., Draves, K. E., Geahlen, R. L., Ledbetter, J. A. & Clark, E. A. (1993) *Proc. Natl. Acad. Sci. USA* 90, 3236–3240.
- Peaker, C. J. & Neuberger, M. S. (1993) *Eur. J. Immunol.* 23, 1358–1363.
- Campbell, M.-A. & Klinman, N. R. (1995) *Eur. J. Immunol.* 25, 1573–1579.
- Doody, G. M., Justement, L. B., Delibrias, C. C., Matthews, R. J., Lin, J., Thomas, M. L. & Fearon, D. T. (1995) *Science (London)* 269, 242–244.
- Lankester, A. C., Van Schijndel, G. M. W. & Van Lier, R. A. W. (1995) *J. Biol. Chem.* 270, 20305–20308.
- Law, C. L., Sidorenko, S. P., Chandran, K. A., Zhao, Z. H., Shen, S. H., Fischer, E. H. & Clark, E. A. (1996) *J. Exp. Med.* 183, 547–560.
- Kitagawa, H. & Paulson, J. C. (1994) *J. Biol. Chem.* 269, 17872–17878.
- Shi, W. X., Chammas, R., Varki, N. M., Powell, L. & Varki, A. (1996) *J. Biol. Chem.* 271, 31526–31532.
- Braesch-Andersen, S. & Stamenkovic, I. (1994) *J. Biol. Chem.* 269, 11783–11786.
- Sgroi, D., Nocks, A. & Stamenkovic, I. (1996) *J. Biol. Chem.* 271, 18803–18809.
- Freeman, S. D., Kelm, S., Barber, E. K. & Crocker, P. R. (1995) *Blood* 85, 2005–2012.
- Collins, B. E., Yang, L. J. S., Mukhopadhyay, G., Filbin, M. T., Kiso, M., Hasegawa, A. & Schnaar, R. L. (1997) *J. Biol. Chem.* 272, 1248–1255.
- Tropak, M. B. & Roder, J. C. (1997) *J. Neurochem.* 68, 1753–1763.
- Powell, L. D., Jain, R. K., Matta, K. L., Sabesan, S. & Varki, A. (1995) *J. Biol. Chem.* 270, 7523–7532.
- Sato, T., Furukawa, K., Autero, M., Gahmberg, C. G. & Kobata, A. (1993) *Biochemistry* 32, 12694–12704.
- Shan, D. & Press, O. W. (1995) *J. Immunol.* 154, 4466–4475.
- Langlois, M., Delanghe, J., Philippe, J., Ouyang, J., Bernard, D., De Buyzere, M., Van Nooten, G. & Leroux-Roels, G. (1997) *Eur. J. Clin. Chem. Clin. Biochem.* 35, 199–205.
- Loftus, J. C., Smith, J. W. & Ginsberg, M. H. (1994) *J. Biol. Chem.* 269, 25235–25238.
- Carrillo, M. B., Milner, C. M., Ball, S. T., Snock, M. & Campbell, R. D. (1997) *Glycobiology* 7, 975–986.
- Guthridge, J. M., Kaplan, A. M. & Cohen, D. A. (1994) *Immunol. Invest.* 23, 393–411.
- Grewal, I. S. & Flavell, R. A. (1996) *Immunol. Rev.* 153, 85–106.
- Clark, E. A. & Ledbetter, J. A. (1994) *Nature (London)* 367, 425–428.